

VITAL STAINING OF THE CENTRIFUGED ARBACIA PUNCTULATA EGG

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The stratification and parts of the *Arbacia punctulata* egg obtained by centrifugal force are shown in Plate I (from E. B. Harvey, 1936). The size of the parts and degree of stratification varies with the centrifugal force; the greater the force, the larger the red half and the less marked the stratification (E. B. Harvey, 1941). In any experimental work with the halves and quarters, it is of importance to know exactly what materials are present. This is best done by the use of vital dyes which stain the different materials differentially.

Table I contains a list of vital dyes used, arranged alphabetically, and the effect of each dye on the various materials in the egg. In all cases, the egg was viable after staining, since it could be fertilized and at least begin development. Different brands of the same dye have been found in some cases to differ considerably both in staining capacity and in toxicity. In general, the dyes put out by the National Medicinal Products or the National Aniline and Chemical Co. gave the best results, but for Nile blue sulphate and thionin, Gröbler's were better.

It was found better to stain the eggs first by allowing them to stand about one-half hour in a dilute solution of the dye in sea water, and then centrifuge them, because the mitochondrial layer disappears in 5-10 minutes after centrifuging and it often takes longer than that for the dye to be taken up by a centrifuged egg. No accurate measure of the amount of dye used was made, but it was soon learned how deeply the sea water should be tinged for the dye to be efficacious but not toxic. Some of the dyes are readily soluble in sea water, others (Bismarck brown, neutral red, Nile blue, safranin O, thionin) must be dissolved in distilled water and a drop of this added to the sea water; some vital dyes (e.g. cresyl violet, Victoria blue) were found not to be sufficiently soluble even in distilled water. No acid dye was found to enter the cell.

The jelly forms a layer around the egg which in *Arbacia punctulata* is 20-30 μ thick. It is invisible under the microscope unless outlined by particles of India ink or stained, since it is of the same refractive index as the sea water. When it is present, the eggs are well separated from each other; when the eggs are contiguous, it means that the jelly

has disappeared, and the eggs are then usually not in optimum condition. The jelly is destroyed by X-rays or by a small amount of acid in the sea water (1 drop of N/10 HCl + 50 cc. of sea water). It is sometimes centrifuged off while the eggs are rotating, though it may remain,

TABLE I
Arbacia punctulata. Vital dyes.

Dye	Jelly	Oil	Clear Layer	Mitochondria	Yolk	Pigment	Remarks
Bismarck brown	0	0	Yellow (upper part more intense)	Yellow	Yellow	Brown	Slightly soluble in sea water
Brilliant cresyl blue	0	0	0	0	Blue	Blue	Very innocuous
Chrysoidin	0	0	Light yellow (upper part more intense)	Light yellow	Yellow	Reddish brown	
Gentian violet	0	0	0	Purple	0	0	
Janus dark blue B	Purple	0	0	0	0	0	
Janus green (=diazin green)	Purple	0	0	Blue	0	0	Rather toxic
Methyl green	0	0	0	Purple	0	0	
Methyl violet	0	0	Upper part violet	Purple	Purple (later)	Purple (later)	
Methylene blue	0	0	0	0	Blue	Blue	Very innocuous
Neutral red	0	0	Pinkish yellow (lower part more intense)	Pinkish yellow	Brick red	Blood red, almost black	Slightly soluble in sea water
Nile blue sulphate	0	0	Light blue (upper part more intense)	Light blue	Blue	Bluish brown to blue black	Slightly soluble in sea water
Rhodamine	0	0	Pink (upper part more intense)	Pink	Pink	Deep red	Very innocuous
Safranin O	Yellow (few cases)	0	0	Pink (after 1-2 hours)	0	Blood red	Not soluble in sea water
Thionin	Pinkish (few cases)	0	0	Lavender (few cases)	0	0	Not soluble in sea water
Toluidin blue	Pinkish lavender	0	Pinkish lavender	Lavender	Lavender	Purple to blue black	More intense if stained after cent.

somewhat elongate, on well centrifuged elongate eggs, or even around the two separated half-eggs when close together, or it may remain around one half-egg. It is best to determine its reaction to dyes on uncentrifuged eggs. The jelly stains *purple* with *Janus green* and *Janus dark blue B*, and *pinkish lavender* with *toluidin blue*; in a few cases it stained *yellow* with *safranin O*, and *pinkish* with *thionin*.

The *oil* cap is not stained by any of the vital dyes. A slight tinge of color was observed in some cases, e.g. with Bismarck brown, chrysoidin and Nile blue, but it is probable that the slight color was in the matrix and not in the oil drops themselves.

The *nucleus* is not stained by any of the vital dyes.

It has been stated that the *clear layer* does not stain in the living egg (Lucké, 1925), and this is certainly true of many dyes. There is no doubt, however, that some dyes do stain the clear layer, not very intensely, while the egg is still living, as could be told by its subsequent development after fertilization. A comparison of the stained egg alongside a control egg in fresh sea water showed whether the clear layer was really stained. The clear layer stains *yellow* with *Bismarck brown* and *chrysoidin*, *blue* with *Nile blue*, *pink* with *rhodamine*, *pinkish yellow* with *neutral red* and *pinkish lavender* with *toluidin blue*. With some dyes there is a decided difference in the intensity of the stain in the upper and lower portions of the clear layer, indicating a stratification of materials within the layer. With Bismarck brown and chrysoidin, which in general act similarly, Nile blue and rhodamine, the upper portion of the clear layer stains more intensely. With neutral red, the lower portion stains more intensely. With *methyl violet*, only the upper portion stains (*violet*). This difference in different regions of the clear layer is more marked when the eggs are stained first and then centrifuged. Although the clear layer is optically empty in the living unstained egg, and no granules can be distinguished in the unstained or vitally stained egg, nevertheless in fixed material, this layer is filled with very fine granules, deeply staining (blue) with Heidenhain's iron hematoxylin (E. B. Harvey, 1940).

The best *mitochondrial* stain is *methyl green*, which stains the mitochondria *purple* and stains no other granules, so that the mitochondria appear as a purple band across the egg. *Gentian violet* also stains the mitochondria differentially (*purple*). *Methyl violet* stains the mitochondria *purple*, like methyl green, but it stains other granules as well. It may be that the purple stain of the methyl green is due to a contamination of this dye with methyl violet or crystal violet, but every brand of methyl green tried has given the same result. *Janus green*, which has been advocated especially by Cowdry as a mitochondrial stain, stains the mitochondria *blue*, but all brands have been found rather toxic, some brands more so than others. *Safranin O* was found to stain the mitochondria *pink* after some time, and *thionin* in a few cases stained them *lavender*. Other dyes stained the mitochondria, but also stained, somewhat more intensely, the underlying yolk (see Table I). As mentioned above, the mitochondrial layer disappears 5-10 minutes after

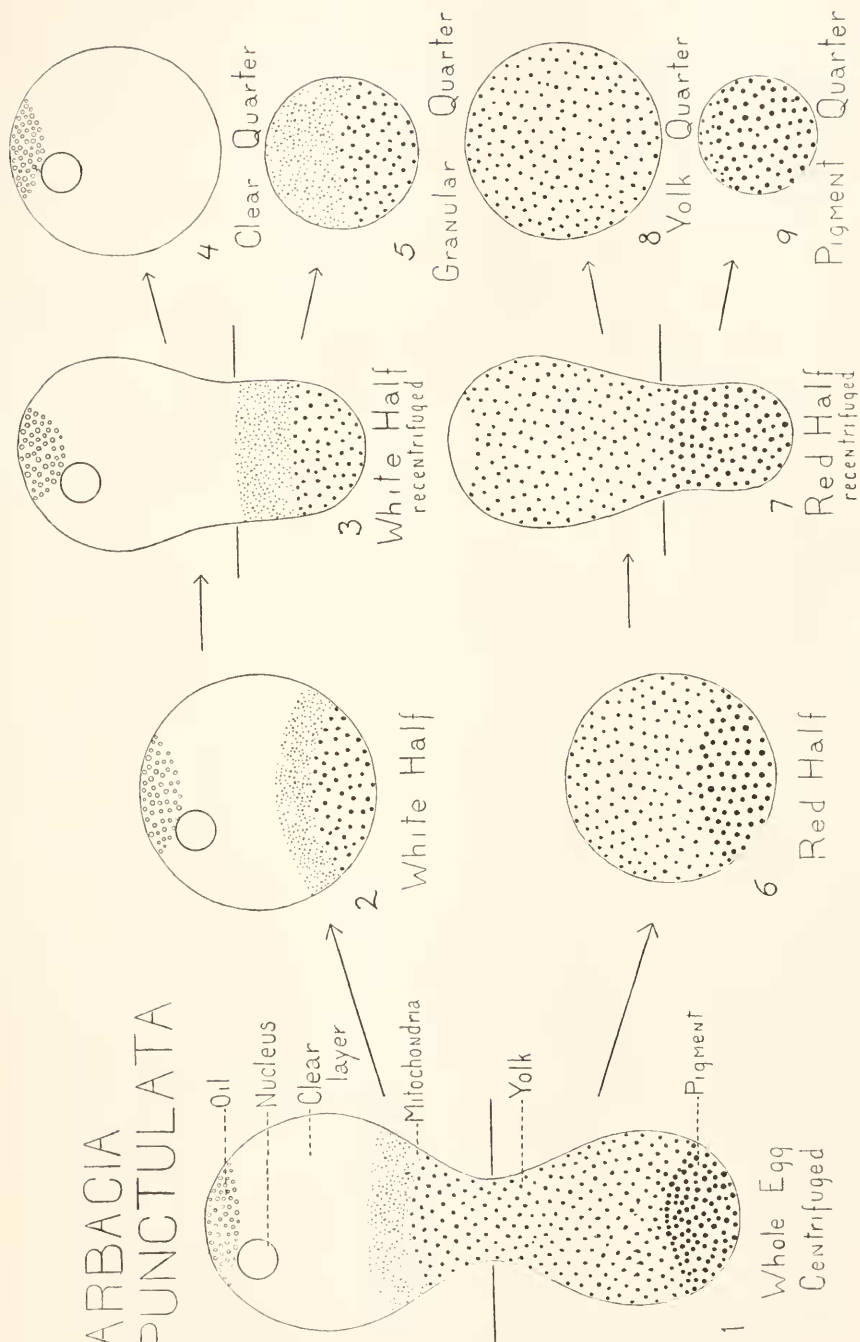


PLATE I

The unfertilized egg of *Arbacia punctulata*, stratified by centrifugal force (about 3 minutes at 10,000 × g.), and the halves and quarters into which it breaks. The drawings are from camera lucida sketches and photographs, made as accurately as possible to scale. Magnified 500 ×.

The clear area in Fig. 7 at the centripetal pole is due to further packing of the granules with longer centrifuging.

removal from the centrifuge, so that observations on it must be made quickly. It is also the last layer to be formed in centrifuging, and in some batches of eggs is not at all sharply defined.

Yolk and *pigment* can be easily distinguished from each other in the unstained egg by the color. They are usually both stained with the same dye, the pigment more intensely and at first more reddish. They are stained *blue* with *brilliant cresyl blue* and *methylene blue*, which in general act alike, and *Nile blue*; *yellow-brown* with *Bismarck brown* and *chrysoidin*; *red* with *neutral red* and *rhodamine*; *lavender-purple* with *toluidin blue*. With *safranin O*, the pigment is stained *blood-red* and the yolk is unstained.

Considerable information as to the chemical structure of the materials in the egg might be obtained from a study of the dye reactions, since the chemical composition of the various dyes is known. For the chemistry and preparation of the dyes and other data, the reader is referred to Rowe's *Color Index* (1924), Schultz' *Farbstofftabellen* (1934) and Conn's *Biological Stains* (1940). For the rate of penetration of the dyes (into gelatin), see Möllendorff's excellent article in *Abderhalden Handbuch der biologischen Arbeitsmethoden*, Abt. V, Teil 2, Heft 2 (1921).

SUMMARY

A table is given of the action of various vital dyes on the different materials in the centrifuged egg of *Arbacia punctulata*. The *jelly* surrounding the egg is stained with Janus green, Janus dark blue B, (purple) and toluidin blue (pinkish lavender). The *clear layer* is slightly stained with Bismarck brown and chrysoidin (yellow), Nile blue (blue), toluidin blue (pinkish lavender), rhodamine (pink), and neutral red (pinkish yellow). The *mitochondrial layer* is differentially stained with methyl green and gentian violet (purple) and Janus green (blue). *Yolk* and *pigment* are stained with brilliant cresyl blue, methylene blue and Nile blue (blue), toluidin blue (purple), rhodamine and neutral red (red), Bismarck brown and chrysoidin (yellow-brown). With safranin, the *pigment* is stained blood red, the yolk is unstained.

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